Evaluation of Efficacy of Microbial Biological Control Agents – Commercially Acceptable Control Levels

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Biological vs. Chemical Paradigm

- Are there different expectations for performance (efficacy, consistency) from biological and chemical control?
- Does placing biological control in a chemical control paradigm hamper its development?
- How the performance level effects cost and use of biological control?
- Can removal of biological control from the chemical paradigm reduce the cost of product development and increase its use?

Cost of Development of Biological Control

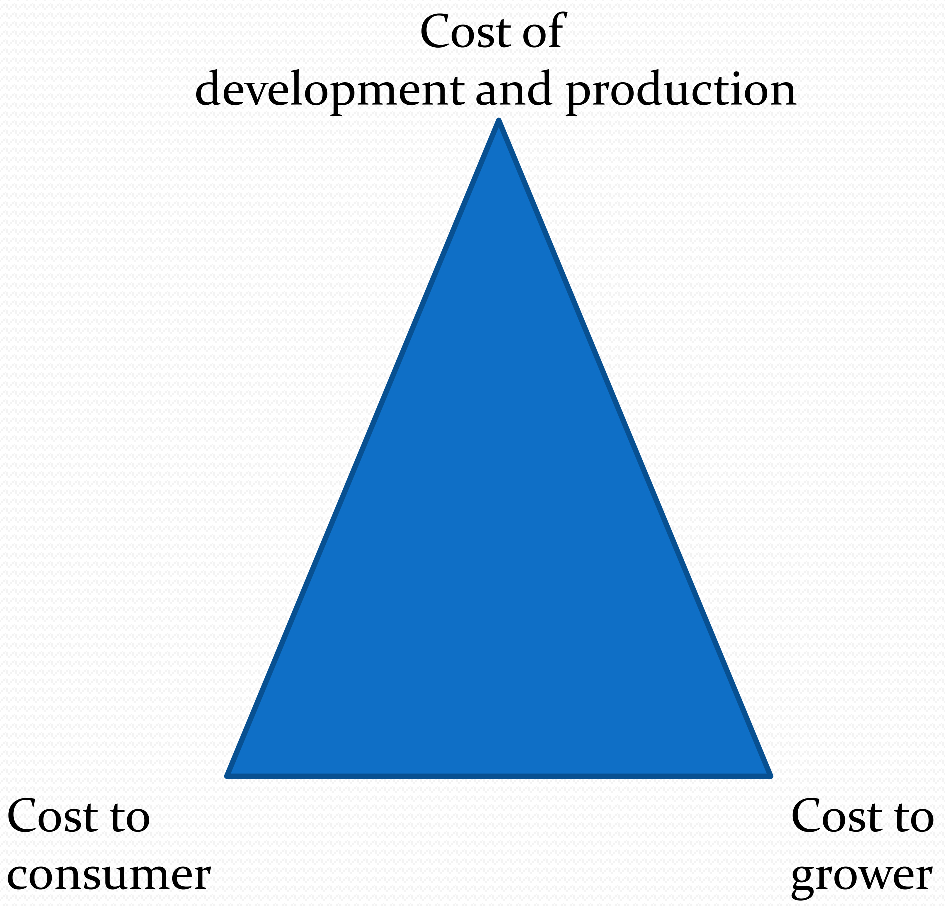
- Chemical model - \$ 8 million
- Biofertilizer, inoculant, or plant strengthening agent model – \$ 1.8 million
- Local production model - \$ 100,000
- Government sponsored/produced agents – cost unknown

After G. Harman et al. 2010. Plant Dis. 94:928-939

Cost, efficacy and the use of biological control are linked together

- Economically driven biocontrol (most commercial products)
- Non-economically driven biocontrol (government sponsored programs)

Cost of biocontrol



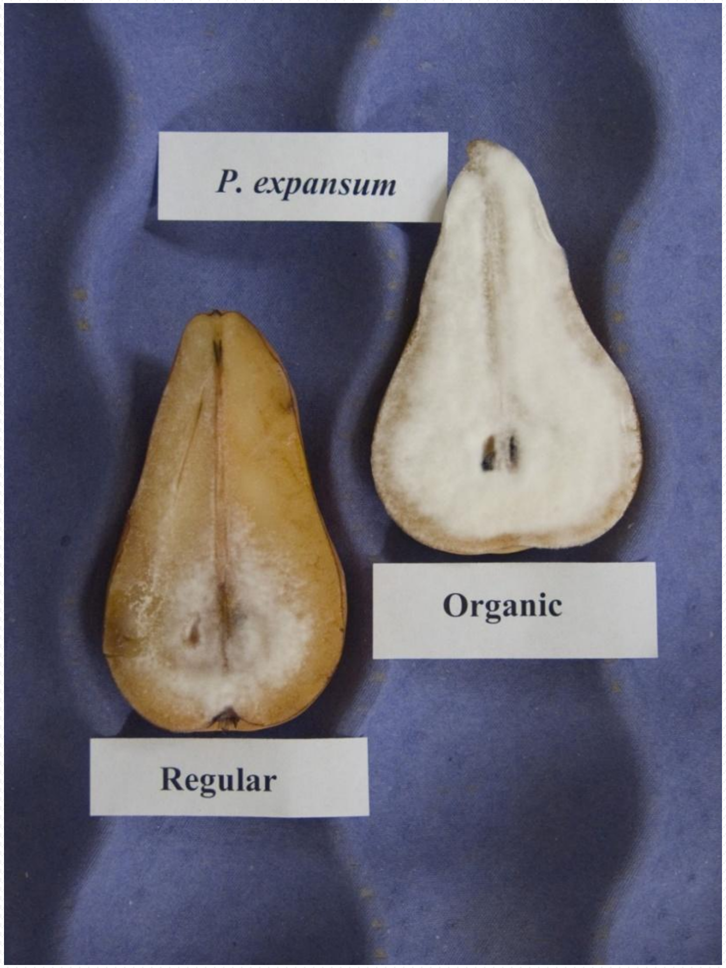
Passing cost to consumers – message the public needs to hear to accept BC

- Pesticide free produce (Consumer concerns about negative effect of pesticide residues EU – 71%, IT – 86%, BE – 55%, NE – 47%, Ben Vorstermans, PCFruit, St-Truiden , Belgium)
- Improved organoleptic (sensory) quality
- Higher nutritional value ?
- Unintended benefits (control of foodborne pathogens)
- Reduced environmental impact


USDA
ORGANIC
ORGANIC
Organic
Apples
POMMES BIOLOGIQUES
NET WT. 3 LBS.
POIDS NET 1.36 kg
PRODUCE OF USA
PRODUIT OF DES É.-U.


Organic Apples, and we wish
you the best of health.
~
We invite you to visit our website:
http://www.gratefulharvest.com
A word or two
about the benefits of
Organic agriculture
What is Organic?
Simply, it's a way of growing fruits and vegetables with materials and practices that build the health of the soil and protect the environment from potential harm caused by synthetic farm chemicals.
Why does Organic produce cost more to grow?
Organic produce sometimes costs more because farmers do not use widely available synthetic chemicals and need to use more expensive efforts to build healthy soil, reduce

Inhibition of *Penicillium expansum* growth on pear flesh tissue by fungicides used in conventional (Regular) production compared to fruit from Organic production, not treated with fungicides



Janisiewicz, unpublished; also see data by Xiao and Boal, 2009. Plant Dis. 93: 1003-1008

Factors affecting acceptable efficacy of biocontrol

- Availability and effectiveness of alternatives
- Consistency of disease control
- Ability to combine BC with other non-fungicidal alternatives
- Return on grower investment
- Value of the commodity
- Production system - added value (conventional, organic, integrated systems)
- Market potential (domestic and export)
- Governmental regulations
    - Registration efficacy data requirement (California, IR-4, Canada, EU for each pathogen/BCA combination)
    - Restriction or ban on using pesticides (Canada, postharvest in Europe)
- Government programs
- Word of mouth about performance and return

> *"We tried different chemicals before, and (Bio-Save 11 LP) works the best for us. The other chemicals we used before, you have to mix them. This is easier to apply, and we've never had any problems with it. It provides good control."*
>
> —STEVE TOBOL
> Seed potato grower of 200 acres, Ronan, Mont.

range, but it seems like an ebb and flow."

Potato growers have become a source of increased sales for Bio-Save in recent years because of fusarium dry rot, a big problem in some U.S. growing areas.

"The potato folks are more interested in moving away from traditional chemicals," Grant says.

One such grower and satisfied Bio-Save 11 LP customer is Steve Tobol, a seed potato grower of 200 acres based in Ronan, Mont. He began using Bio-Save 11 LP in the last few years for fusarium dry rot based on the recommendation from another grower.

"We tried different chemicals before, and (Bio-Save 11 LP) works the best for us," Tobol says. "The other chemicals we used before, you have to mix them. This is easier to apply, and we've never had any prob-

Examples of efficacy of biological control products

- **In the field**

  Above ground diseases (e.g. Blightban, Afla-guard)

  Soil-borne diseases (suppressive soils, nematodes, damping-off system)

- **Controlled environments**

  Postharvest (BioSave)

Biocontrol of fire blight in pear orchards

Incidence of Fire Blight Strikes in a Commercial Pear Orchard Sprayed with Antibiotics At Different Frequencies and Also Treated with *Pseudomonas fluorescens* strain A506

Treatment	Infections/Acre
50% Antibiotic Frequency	30.7 a
50% Antibiotic Frequency + A506	10.2 b
100% Antibiotic Frequency	9.8 b
100% Antibiotic Frequency + A506	2.4 b

Severity of Fruit Russeting and Incidence of Frost Damage at Harvest on Pear Trees Treated with Antibiotics at Different Frequencies and also Treated with *Pseudomonas fluorescens* strain A506 (Blightban A506)

Treatment	Fruit Russet (% of Surface)	Frost Damage (Fraction of Fruit)
50% Antiobiotics - No A506	2.6 a	0.26 a
100% Antibiotics - No A506	2.4 a	0.20 a
100% Antibiotics + A506	1.3 b	0.07 b
50% Antibiotics + A506	1.2 b	0.08 b

Economic analysis of costs associated with fire blight, fruit russet, and frost control by applying Blightban A506 multiple times in water alone or a single time with penetrating surfactants

	Standard Program (3 sprays at 100% dose)	Every other row Standard Program (6 sprays at 50% dose)	Early Season Penetrant Program (0.5% Breakthru)	Early Season Penetrant Program (.25% Breakthru)
Blightban A506	36.5 x 3 = 110	18.25 x 6 = 110	36.5 x 1 = 37	36.5 x 1 = 37
Breakthru	0	0	50	25
Spraying Costs	30 x 3 = 90	20 x 6 = 120	30 x 1 = 30	30 x 1 = 30
# of Sprays	3	6	1	1
Total Cost $/acre	**200**	**220**	**117**	**92**

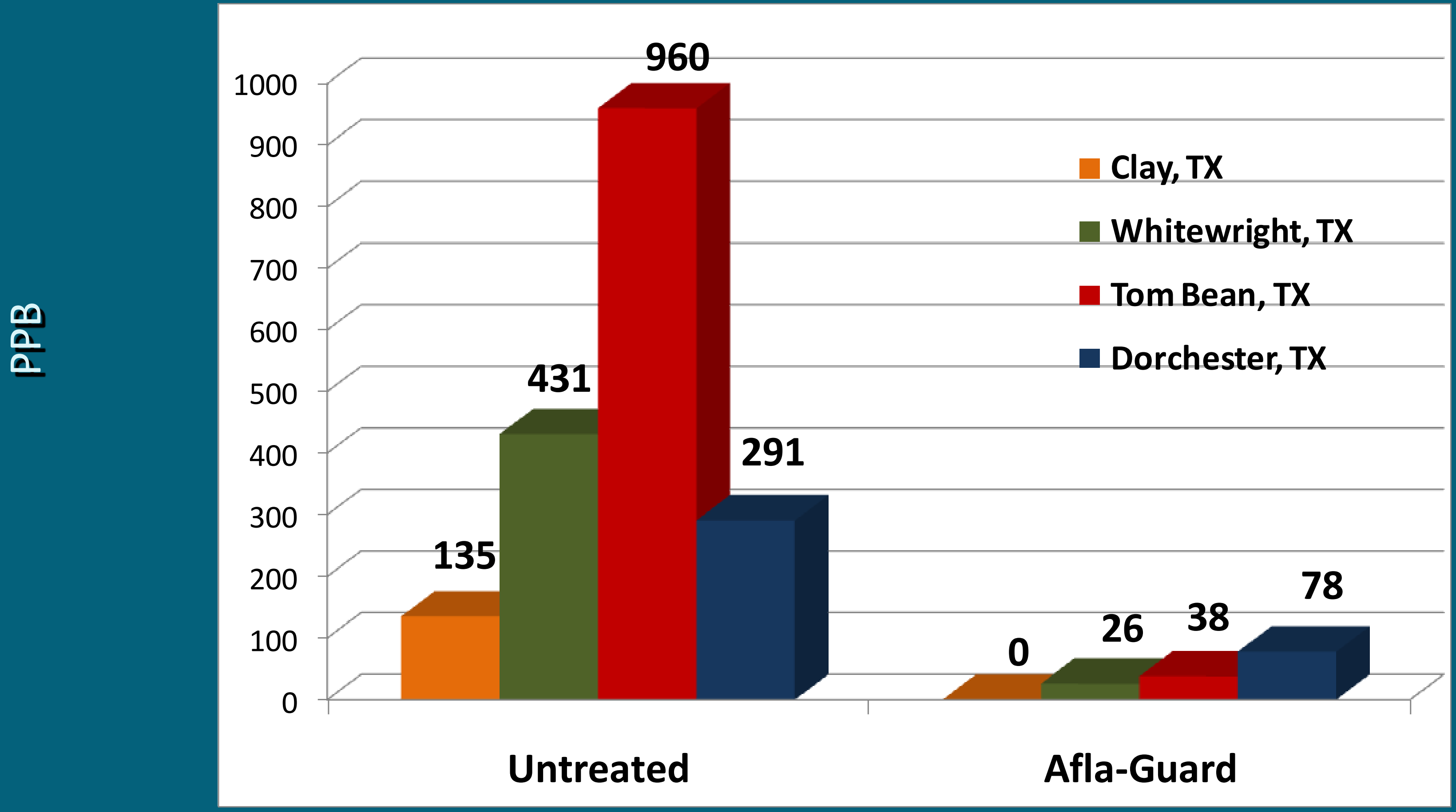
Assumptions:
Breakthru = $100/gallon
Blightban A506 = $76/10.5 oz
Blightban used at 5 oz/acre
Spray volume = 100 gal/acre
Sprayer costs = $30/acre for every row
= $20/acre for every other row
Assume that Blightban applied in dedicated sprays (except with Streptomycin)

Acceptable level of biocontrol:

number of fire blight strikes and fruit russet comparable to antibiotic treatment

Reduction of Aflatoxin by Biological Control Agents

Effect of Afla-Guard on aflatoxin concentrations (ppb) corn - large acre grower fields (2009)



Effect of Afla-guard on aflatoxin level (µg/kg) in commercial farmers' stock peanuts

Location	Untreated	Treated	% Reduction
Hartford, AL	16.6	5.5	
Newton, AL	319.7	49.0 ***	**84.7**
Ft. Gaines, GA	96.6	0.2 ***	**99.8**
Sasser, GA	0.0	0.0	
Smithville, GA	0.0	0.1	
Unadilla, GA # 1	37.4	0.0 **	**100.0**
Unadilla, GA # 2	2.6	1.0	
All Locations	78.9	11.7 ***	**85.2**

** $P < 0.01$; *** $P < 0.001$

Acceptable level of biocontrol: any reduction in aflatoxin, preferably to less than 20 ppb (set by FDA)

Nonchemical Alternatives for control of Peach Tree Short Life (PTSL)

Biological Control

Solarization

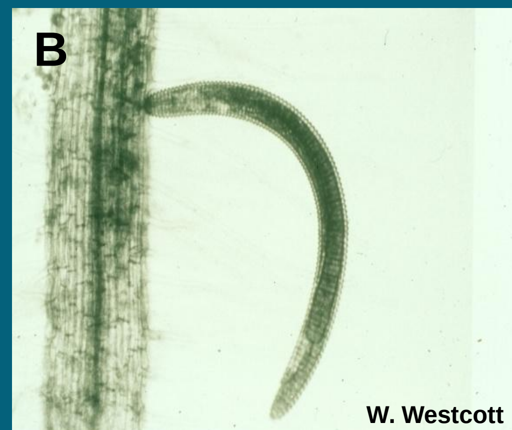
Tree Survival (%) on PTSL Site
(2000-05)

	Guardian	Nemaguard
NF	83 a	16 b
MBR	90 a	12 b

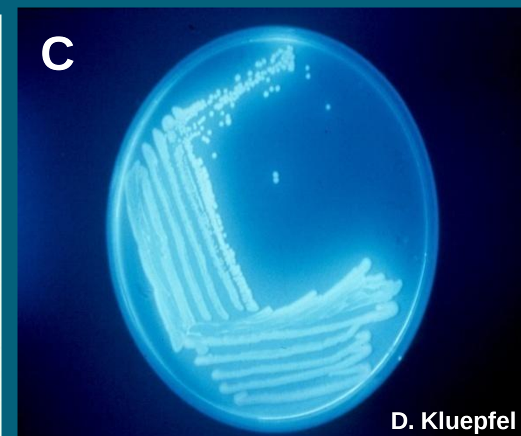
The 6 dead trees in the foreground are on Nemaguard rootstock (ring nematode & PTSL susceptible) and the 6 living trees in the same row are on Guardian rootstock (ring nematode & PTSL tolerant).

Ring Nematode Suppressive Soil Detected in SC

PTSL Site

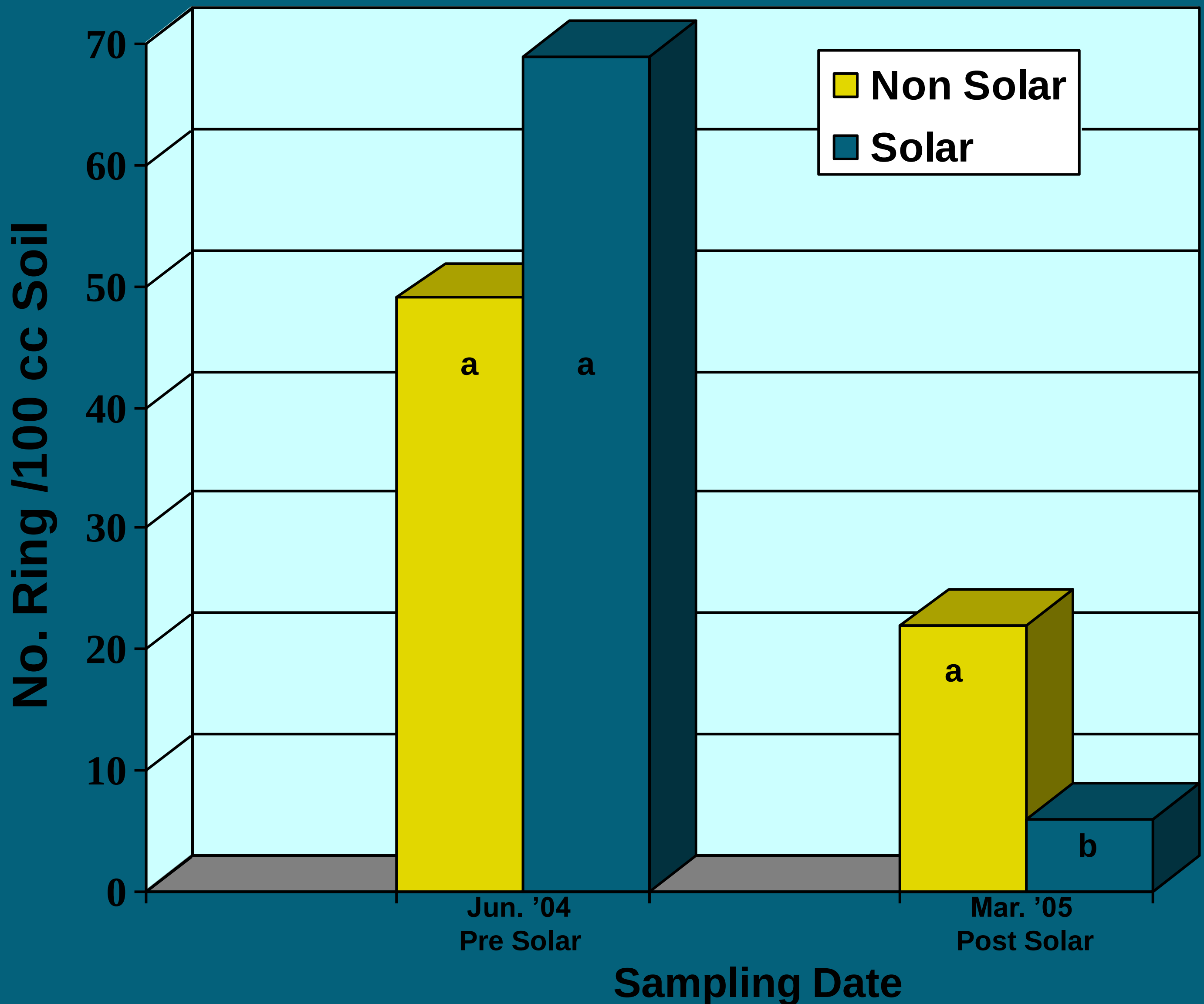


Ring Nematode



P. synxantha (BG33R)

Ring Populations as Influenced by Solarization



Biological Control of Ring Nematode

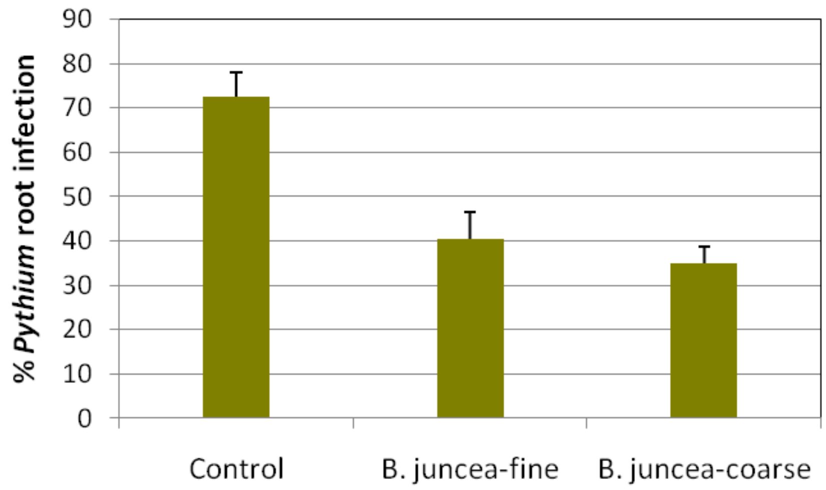
Applying BG33R thru Irrigation System

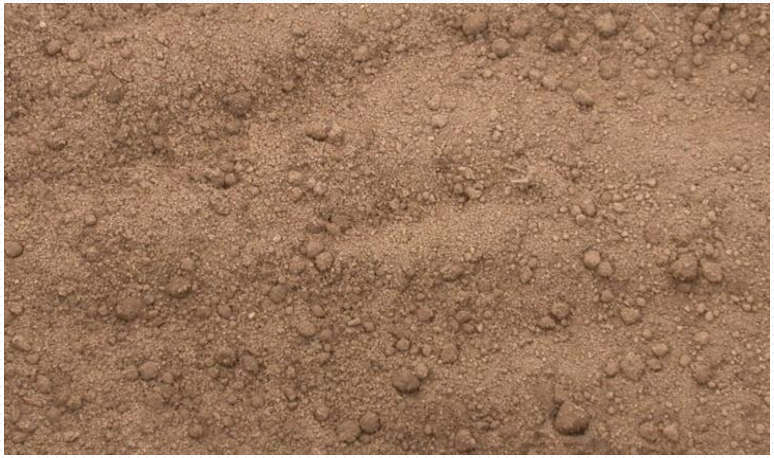
D

	Trunk Diameter (36 mo.)	
1.	Solar + BG33R	77.89 a
2.	Solar	72.14 ab
3.	No Solar + BG33R	76.15 ab
4.	No Solar	69.06 b

Apple replant

Long-term biological suppression *Pythium* induced by *B. juncea*



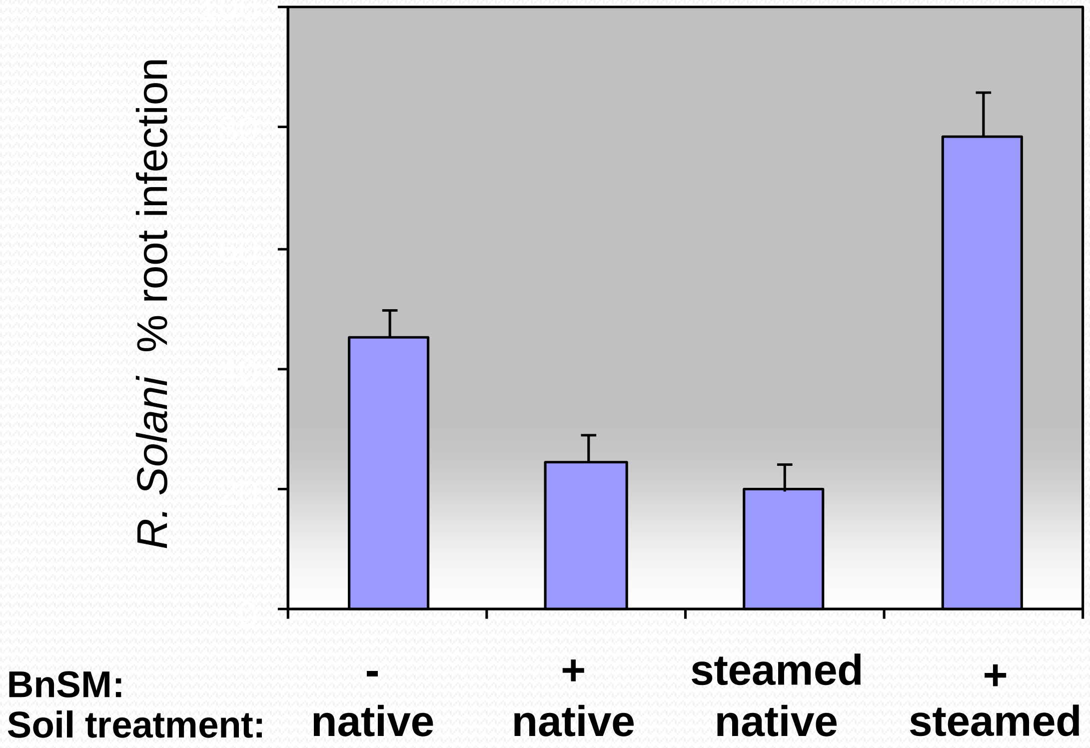


Control

B. juncea

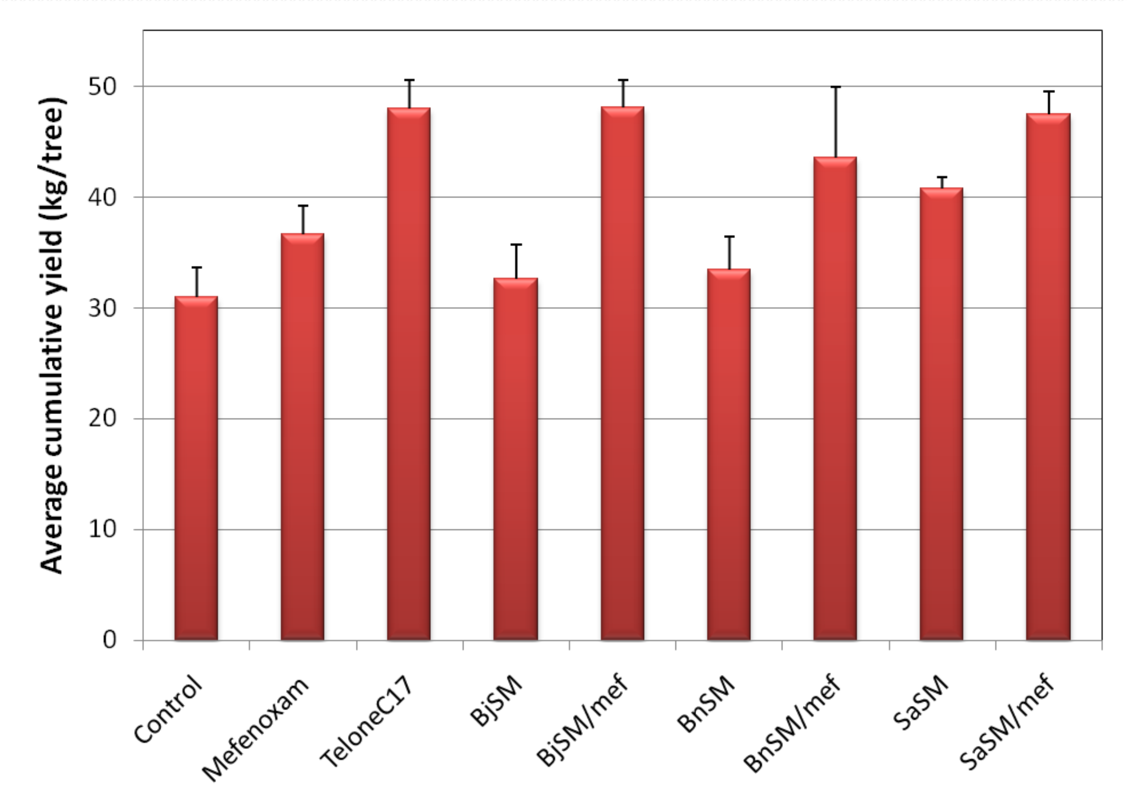
Control of *R. solani* with *B. napus* seed meal (BnSM) amendment

A functional soil microbial community is required for suppression



Cohen and Mazzola 2005; 2006

Impact of soil treatments on Gala/M26 yield 2006-2009



BjSM=Brassica juncea seed meal
BnSM=Brassica napus seed meal
SaSM=Sinapis alba seed meal
mef=mefenoxam

Mazzola and Brown, 2010

Acceptable level of biocontrol:

PTSL – nematode control for two years after preplant application, and extension of peach tree life to 15+ years

Apple replant – yield equivalent to fumigated plots

Biocontrol Treatments Promoting Seedling Stand

Suppression of damping-off of cucumber caused by *Pythium ultimum* using seed treatments with *Enterobacter cloace* strains

Treatment	% Stand	Plant height (mm)
Healthy control	99 A	53.5 A
501R3	93 A	50.3 ABC
S17R1	97 A	55.5 A
501R3 + *P. ultimum*	93 AB	45.0 BCD
S17R1 + *P. ultimum*	92 AB	44.3 CD
Control (*P. ultimum)*	30 C	20.1 E

Roberts et al. (1997). J. Phytopathol.

Acceptable level of biocontrol:

increasing seedling stand to the level of uninoculated control

Biological Control of Postharvest Diseases of Fruits and Vegetables

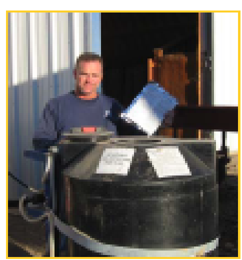
What is Bio-Save?

- Bio-Save is an EPA registered post harvest biological decay control agent. Biological control is defined as the use of natural organisms to reduce the effects of pests and disease.
- Bio-Save is a freeze-dried formulation of bacterium. Freeze-drying allows for easy storage. Bio-Save comes in easy to handle foil pouches.
- Bio-Save's main ingredient is the formulated bacterium, pseudomonas syringae.
- Bio-Save is an approved organic product listed with

OMRI Listed
Organic Materials Review Institute

Pseudomonas Syringae

- *Pseudomonas syringae* is a vegetative microorganism. This bacterium allows for fast growth and works under competitive inhibition.
- *Pseudomonas syringae* (formulated as Bio-Save) enters the wound where the mold spores (disease) is located and (at storage temperatures) will last in the wounds throughout the storage period.
- Once in the wound the pseudomonas bacterium (Bio-Save) cannot be killed by surface sanitizers.



Applications for Bio-Save

Bio-Save is labeled for post harvest applications for the control and prevention of several fruit and vegetable diseases including:

- *Fusarium Dry Rot*
  Silver Scurf
  for the treatment of **Potatoes**
- *Rhizopus Soft Rot*
  for the treatment of **Sweet Potatoes**

RHIZOPUS SOFT ROT

- *Penicillium Blue Mold*
  Penicillium Green Mold
  Geotrichum Sour Rot
  for the treatment of **Citrus (lemons, oranges and grapefruit)**
- *Penicillium Blue Mold*
  Botrytis Gray Mold
  Mucor Rot
  for the treatment of **Apples and Pears**

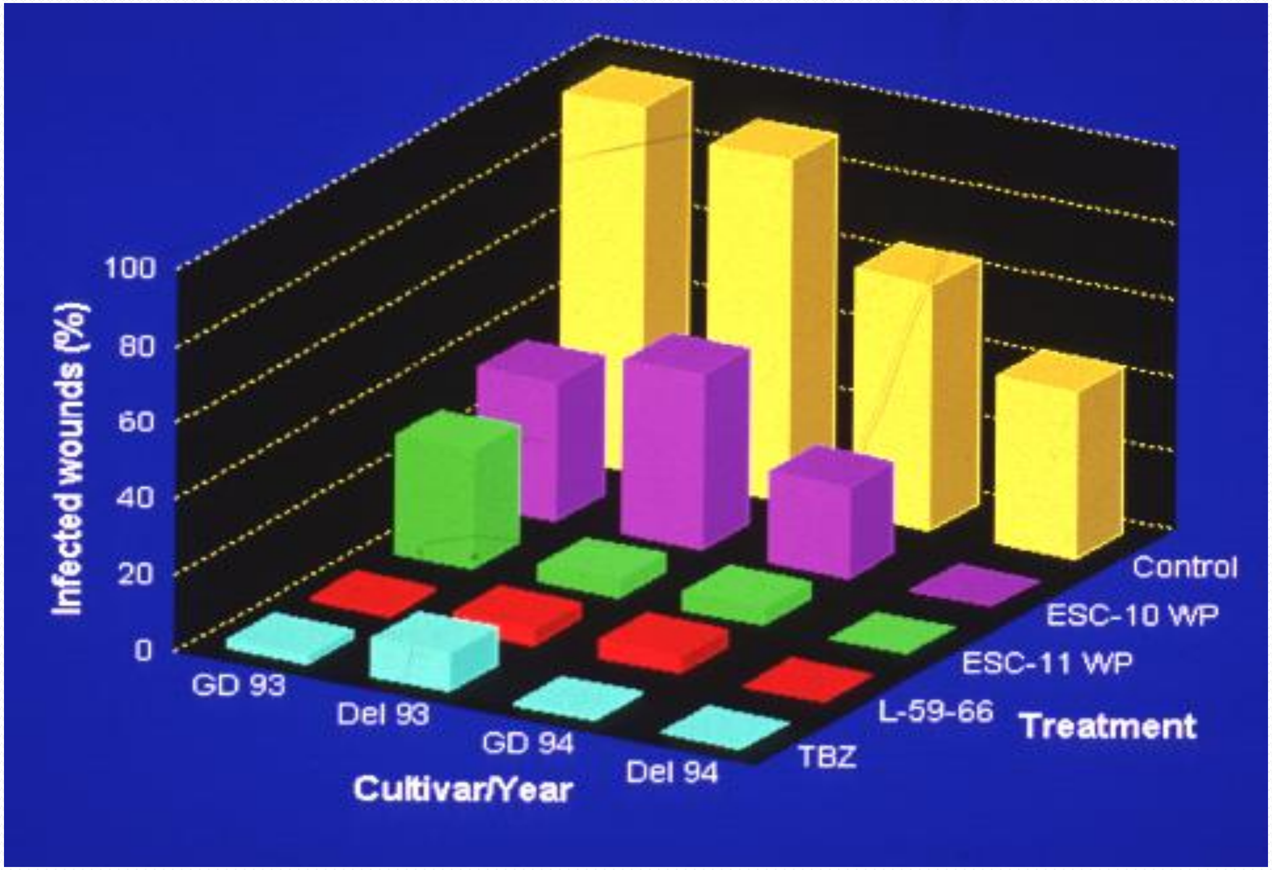
- *Penicillium Blue Mold*
  Botrytis Gray Mold
  for the treatment of **Cherries**

Treatment with Bio-Save can be used alone or in subsequent treatment with other surface sanitizers to synergistically increase effectiveness of both compounds.

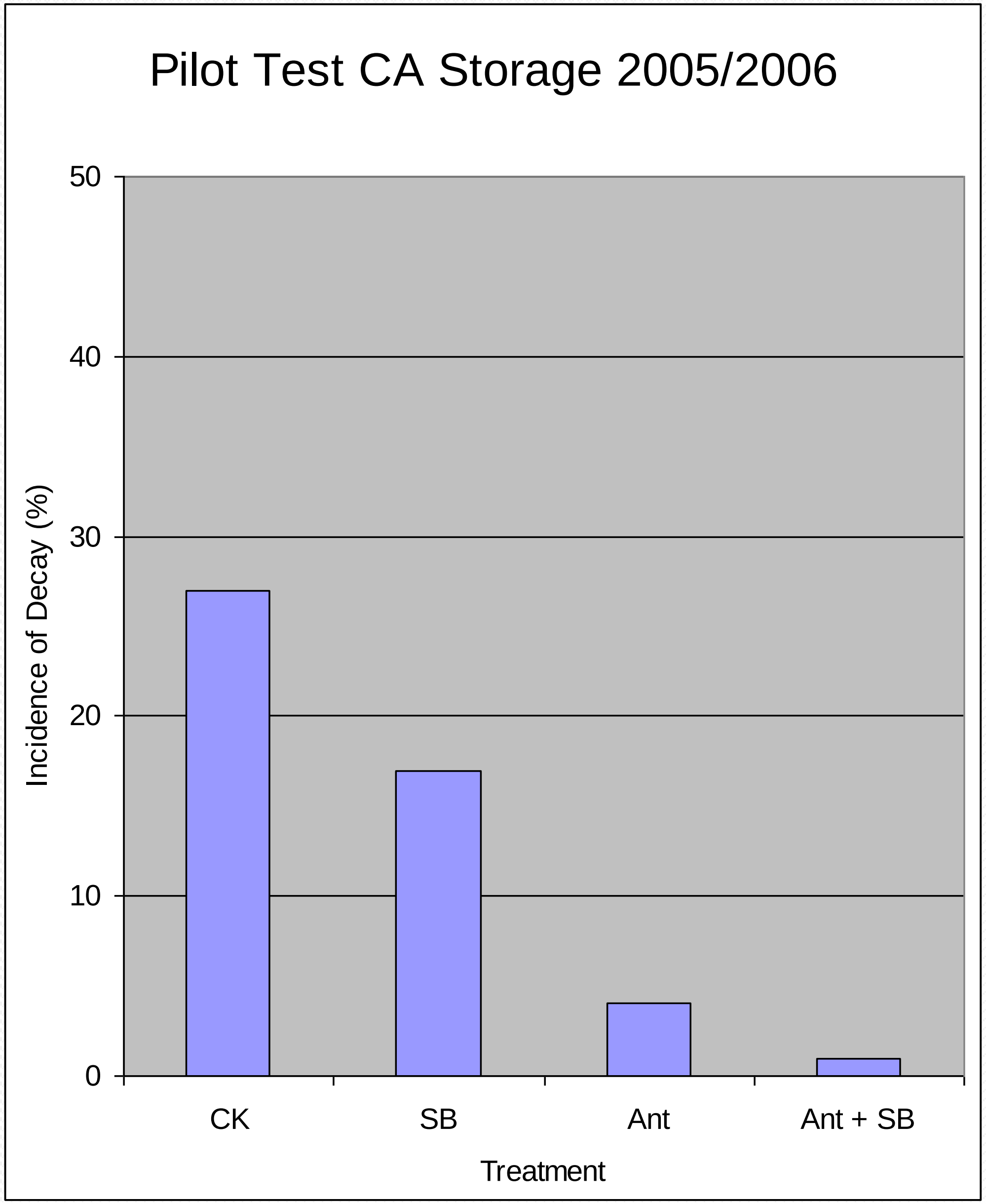
Never directly mix Bio-Save with other sanitizers.


CONTROL
2.2 X 10^8
3.2 X 10^8
5.4 X 10^8
L-59-66 CFU/ML
COLD STORAGE 30 DAYS
P.EXPANSUM
BOSC

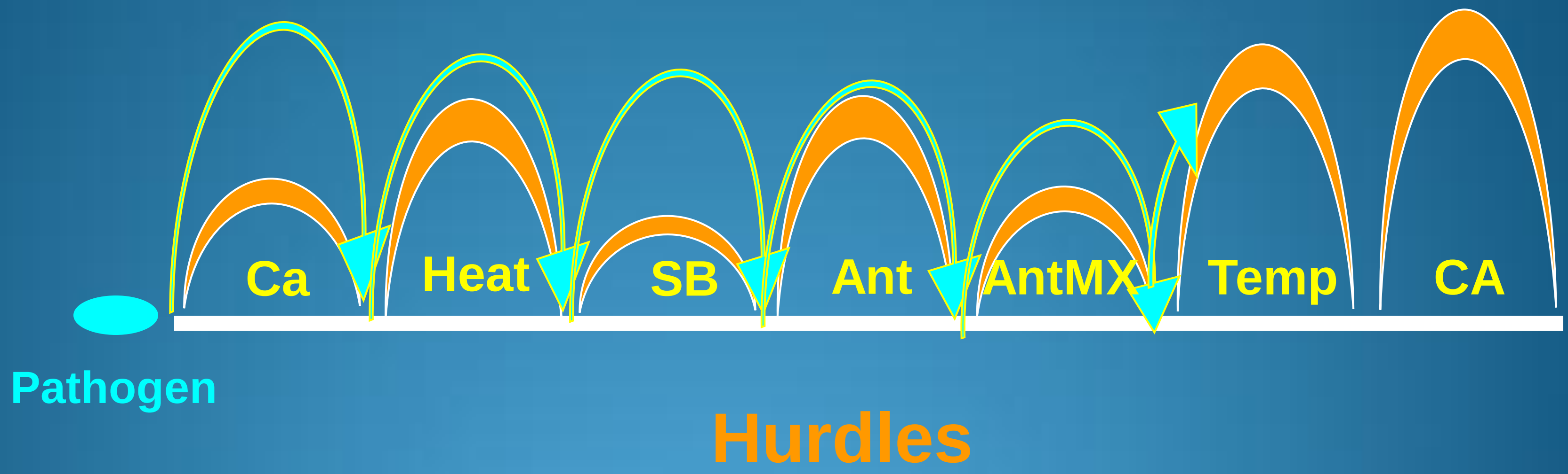
Pilot Test at Kearneysville



Monitoring antagonist populations


Pilot Test CA Storage 2005/2006
Incidence of Decay (%)
0
10
20
30
40
50
CK
SB
Ant
Ant + SB
Treatment

Hurdle Concept (Leistner 1978)



Ca = calcium, Heat = 38C for 4 d, SB = sodium bicarbonate, Ant= antagonist, AntMX = antagonist mixture; Temp = low storage temperature, CA = controlled atmosphere storage

Acceptable level of biocontrol: **consistent reduction of natural fruit decay (3-9%) by half for organic fruit; keeping decay below 2% for fruit in conventional production** (Charlene Jewell JBT Food Tech, Riverside, CA)

Opportunities for increasing efficacy and consistency of biocontrol systems

- **Using locally adopted strains** (more strains for registration)
- **Enhancing biocontrol performance** (combining antagonists, nutritional additives)
- **Combining biocontrol with other alternative control treatments**
- **More field tests** (under commercial conditions, e.g. heat tunnels example)
- **Improving formulations** (shelf life)
- **Quality control of biocontrol products**

What is acceptable level of biocontrol?

It depends on how much market will tolerate for each individual crop

Acknowledgment

- Steve Lindow - University of California, Berkeley, CA – biocontrol of fireblight
- Bruce Horn – USDA-ARS National Peanut Res. Lab., Dawson, GA - biocontrol of aflatoxin,
- Andy Nyczepir – USDA-ARS Southeastern Fruit & Tree Res. Lab., Byron, GA, - biocontrol of peach tree short life
- Mark Mazzola – USDA-ARS Tree Fruit Res. Lab. – biocontrol of apple replant